Final Report-Teratologic Evaluation of FDA 71-38 (**Methyl paraben**) in Mice, Rats, Hamsters & Rabbits 12/1/72 MRO

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Teratologic Evaluation of FDA 71-38

(Methyl Paraben)

(16)

in

Mice, Rats, Hamsters and Rabbits

MONOGRAPH
ON
METHYL PARABEN
AND
PROPYL PARABEN

TR-72-1552-08

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METHYL PARABEN

and

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Summary

In a study by Matthews et al. in which dogs were given, orally, 1.0 g methyl or propyl paraben/kg body weight/day for one year, between 96% and 100% of the daily administered dose was accounted for in the urine, chiefly in the form of conjugated end products. They observed that the parabens are readily and completely absorbed from the gastrointestinal tract (162).

Schuebel and Manger reported that the first step of paraben detoxication is saponification, resulting in formation of PHBA. They suggest that most of this acid is decomposed in the body, the benzene ring being broken, while the rest is eliminated through the kidneys in the form of salts or as free acid. They found no evidence of accumulation of the parabens or their metabolites in the animal body (262).

Jones et al. found no accumulation of methyl or propyl paraben or their metabolites in dogs which had been given 1.0 g/kg/day for one year. They were able to account for 96% of the daily administered dose in the urine in the form of free or conjugated PHBA. In vitro studies using esterases found in the liver and kidneys implicated those organs as the sites of paraben detoxication. By giving a fasted man 70 mg of methyl paraben/kg body weight, they determined that the paraben is handled similarly in human metabolism (136).

In a study by Sabalitschka and Neufeld-Crzellitzer in which they gave a human subject 2 g of propyl paraben daily for 5 days, they found that 17.4% of the administered dose was excreted in the form of PHBA. They computed that about 55% of the paraben was excreted paired with sulfuric acid. It was suggested that breaking of the benzene ring takes place to some extent during detoxication (249).

Nathan and Sears reported that methyl paraben, when applied directly to the nerve, blocks nerve conduction (180).

Bubnoff et al. found that i.v. and orally administered methyl paraben exhibits an anticonvulsive effect in rats with cocaine-induced cramps (39).

There have been relatively few feeding studies on the toxic effects/levels of the paraben esters, either methyl or propyl; although there is quite a bit of data available on the acute toxicity of these compounds. This body of scientific literature is unique in two senses: first, it is primarily German and Italian; second, the literature entries are cross-referenced and all too frequently quoted without citation so that despite the large number of entries in the literature, there is really very little information available.

Short-term feeding studies have been undertaken with methyl paraben on rats, rabbits, cats, dogs, and man; similar studies involving propyl paraben have been conducted on rabbits, dogs, and man. Long-term studies involving both of these esters have been conducted with rats as test subjects.

Bijlsma reported in 1928 on growth depression in rats fed methyl paraben at 200 and 500 mg/kg/day for an unspecified period; however, he was unable to repeat these results (24). Schuebel and Manger reported that rabbits withstood 500 mg of methyl paraben/kg/day for 6 days; but, when this level was raised by 1000 mg/kg per day, the rabbits were affected by the 9th day at a level of about 3000 mg/kg/day (262). In contrast to this, they mentioned that a cat fed methyl paraben at 500 mg/kg/day for 6 days became ill immediately upon challenge and remained so for the duration of the dosage (262). Bijlsma found that a dog fed methyl paraben at 105 mg/kg/day for 4 days was unaffected (24), and Schuebel and Manger found that this dosage could be increased to 500 mg/kg/day for 7 days with no effect on dogs (262); however, when this edosage was increased to 2000-3000 mg/kg/day, the dogs were dramatically affected (262). Matthews, et al., in the definitive short-term study on methyl paraben showed that dogs fed 500 and 1000 mg/kg/day were unaffected after 1 year on the regimen (162). Sabalitschka, et al., report that a man was unaffected after taking 2000 mg of methyl paraben daily for 1 month (249); Bracessi confirmed these observations (35).

Schuebel and Manger gave rabbits 500 mg of propyl paraben/kg/day for 6 days, and these animals showed no ill effects (262). However, at a level of 3000 mg/kg/day, there was evidence of toxicity (262). They also reported that dogs showed ill effects only at levels of 400 mg of propyl paraben/kg/day and higher (262). Matthews, et al., reported that propyl paraben fed at dosages of 500 and 1000 mg/kg/day for 1 year to dogs resulted in no untoward effects (162). Sabalitschka, et al., report that a man was unaffected after taking 2000 mg of propyl paraben daily for 1 month (249).

Long-term feeding of both methyl and propyl paraben to rats by Matthews, et al., revealed that, at dosage of 1000 mg/kg/day for 96 weeks, neither methyl nor propyl paraben showed toxicity for rats (162). At a level of 4000 mg/kg/day for 96 weeks, both esters definitely depressed the growth rate of rats; although the depression was more noticeable in the male rats (162). Sokol reported that propyl paraben stimulated growth in rats when fed at a dosage of 150 mg/kg/day for 18 months, but at a dosage of 1500 mg/kg/day for the same period, a growth depression without specific pathology resulted (272).

In a report submitted by the Heyden Chemical Company, it was found that a mixture of paraben esters (60% propyl - 40% ethyl) fed to rats over a period of 18 months at levels of 14.3 and 143 mg/kg/day resulted in a slight growth increase. At a level of 1430 mg/kg/day for 18 months, this same mixture depressed growth from month 4 to month 8; however, over the entire period of the study, growth of control and test rats was comparable at this level (8).

Boyland and Homburger both report that methyl paraben does not exhibit carcinogenic activity (34, 121). Matthews, et al., failed to show that guinea pigs became sensitized to either methyl or propyl paraben, but they did show that methyl paraben solutions in propylene glycol exerted an irritant action at a concentration above 5%; propyl paraben showed the same effect at concentrations above 12% (162).

There have been several reports in the literature on paraben dermatitis. The consensus seems to be that these paraben esters can cause a dermatitis in susceptible individuals which may be diagnosed with great difficulty as a result of the cross-reactivity of the parabens. Reportedly, this situation is more common in Europe where the parabens are permitted in higher concentrations (260, 255, 83).

B. Food Grade
See C

C. Food Chemicals Codex

Assay	Not less than 99% $C_8H_8O_3$
Melting Range	125° and 128°
Limits of Impurities	
Acidity	Passes Test
Arsenic (as As)	Not more than 3 ppm
Heavy metals (as Pb)	Not more than 10 ppm
Residue on ignition	Not more than 0.05%

VI. Description

A. General Characteristics

Small, colorless crystals or a white, crystalline powder. It is odorless or has a faint characteristic odor and has a slight burning taste.

B. Physical Properties

Melting point	125° - 128°
Boiling point	270° - 280°

Solubility

Solvent	g/100 g solvent
Water, 25°C	0.25
Water, 15°C	0.16
Water, 80°C	3.2
Ethanol, 25°C	52.0
Ethanol, 10%, 25°C	0.5
Ethanol, 50%, 25°C	18.0
Propylene glycol, 25°C	22.0
Propylene glycol 10%, 25°C	0.3
Propylene glycol 50%, 25°C	2.7
Glycerin, 25°C	1.7
Peanut Oil, 25°C	0.5
Ether	14.2

Methyl paraben is also soluble in acetone, slightly soluble in fixed oils, benzene, and carbon tetrachloride.

C. Stability

Methyl paraben should be stored in tight containers. Since esters of p-hydroxybenzoic acid are hygroscopic, their containers should be stored in a dry place.

VII. Analytical Methods

Genest and Chapman developed a method for extracting preservatives from various foods. Liquid-liquid extraction is performed with chloroform, evaporated and dissolved in ethanol. The solution is then analyzed by paper chromatography. Methods are described for extraction from catsup,

METHYL PARABEN

Chemical Information

I. Nomenclature

A. Common Name
Methyl paraben

B. Chemical Names
1. Methyl p-hydroxybenzoate
2. p-Hydroxybenzoic acid methyl ester

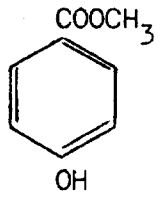
C. Trade Names
1. Methyl parasept
2. Nipagin M
3. Tegosept M

D. Chemical Abstracts Services Registry Number
000099763

II. Empirical Formula

$C_8H_8O_3$ $(CH_3OOCC_6H_4OH)$

III. Structural Formula



IV. Molecular Weight

152.14

V. Specifications

A. Chemical (USP)

Appearance	Small crystals, or white powder
Odor	Faint, Characteristic
Assay (dry basis)	99% Minimum
Melting Range	125°C - 128°C
Acidity	Slightly acid to litmus
Loss on drying (5 hrs. over silica gel)	0.5% Maximum
Residue on ignition	0.05% Maximum
Chloride	350 ppm Maximum
Sulfate	200 ppm maximum

jellies, jams, fruit drinks, margarine, cheese, and preserved fish (101).

Hoyem developed a method using paper chromatography and ultraviolet spectrophotometry. The spotted filter paper is placed in a chromatography tank containing n-butanol, ammonia and water (5:2:3) for 20 hours. The paper is then exposed to an ultraviolet lamp for development of the spots. This procedure can be quantitated by eluting the spots with either 0.01 N HCl or 0.01 N NaOH and measuring at Lambda-max on a UV spectrophotometer (124).

Thin-layer chromatography (TLC) has been extensively used to separate and identify the parabens. Rangone and Ambrosio used silica plates and developed them in borate buffer, adding a given volume of organic solvent as required. The following buffer-solvent combinations have proved most suitable for separation of the various preservatives; buffer-methyl alcohol (90:10), buffer-ethylcellosolve (90:10), buffer-ethyl acetate (saturated solution at 25°), buffer-ethyl ether (saturated solution at 15°), buffer-tetrahydrofuran (95:5), buffer-dioxane (90:10). The spots were then analyzed using a 250 millimicron UV source. The spots were removed with methanol using a vacuum technique and were than quantitated using UV spectrophotometry. The coefficient of variation was 3% for the straight-line determination (211). Another TLC system has been reported with polyamide-silica gels used as the stationary phase and solvents such as n-hexane-benzene-glacial acetic acid (1:1:1), water-28% ammonia solution (20:5). This method can be used to identify the parabens, sorbic acid, and dehydroacetic acid (49).

Gas chromatography has been used to detect methyl p-hydroxybenzoate and separate it completely from a mixture of sorbic acid, benzoic acid, salicylic acid, dehydroacetic acid, menadione and ethyl, butyl, and propyl p-hydroxybenzoate without any pretreatment. The column was packed with 30% DC 550 silicone on celite 545, the column temperature was 190° and the carrier gas was hydrogen (127).

Parabens present in urine and body tissues may be determined by a method using 4-amino-antipyrine. A buffered solution of the sample was centrifuged under conditions appropriate to the type of sample, 4-amino-antipyrine and potassium ferrocyanide. The color formed was measured at 490 millimicrons (136). This method was simplified by Inamine, Obatake and Matsuda (128).

VIII. Occurrence

Methyl paraben is produced by esterifying p-hydroxybenzoic acid with methanol, using an acid catalyst such as sulfuric acid and an excess of methanol. The materials are heated in a glass lined reactor under reflux. The acid is then neutralized with caustic soda, and the product crystallized by cooling, centrifuged, washed, dried under vacuum, milled and blended, all in corrosion resistant equipment to avoid metallic contamination.

PROPYL PARABEN

Chemical Section

I. Nomenclature

A. Common Name
Propyl paraben

B. Chemical Names
1. Propyl p-hydroxybenzoate
2. p-Hydroxybenzoic acid propyl ester

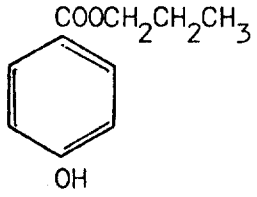
C. Trade Names
1. Nipasol
2. Chemocide PK
3. Propyl Chemosept
4. Solbrol P
5. Propyl Parasept

D. Chemical Abstracts Service Registry No.
000094133

II. Empirical Formula

$C_{10}H_{12}O_3$

III. Structural Formula



IV. Molecualr Weight

180.20

V. Specifications

A. Chemical USP

Appearance	small crystals, or white powder
Odor	none
Assay	99% minimum
Melting range	95-98 deg. C
Acidity	acid to litmus
Loss on drying (5 hrs. over silica gel)	0.5% maximum
Residue on ignition	0.05%
Chloride	350 ppm maximum
Sulfate	200 ppm maximum

B. Food

See A above

C. Food Chemicals Codex

Assay	Not less than 99% $C_{10}H_{12}O_3$
Melting range	Between 95° - 98°
Limits of Impurities	
Acidity	Passes test
Arsenic	Not more than 5 ppm (0.0003%)
Heavy metals (as Pb)	Not more than 10 ppm (0.001%)
Residue on ignition	Not more than 0.05%

VI. Prescription

A. General Characteristics

Propyl paraben appears as small, colorless crystals or a white powder.

B. Physical Properties

Melting point 96-97°

Solubility Data

Solvent	Solubility (g/100 g solvent)
Water, 25°C	0.04
Water, 15°C	0.023
Water, 80°C	0.45
Water, 100°C	2.5
Ethanol, 25°C	95.0
Ethanol, 10%, 25°C	0.1
Ethanol, 50%, 25°C	18.0
Propylene Glycol, 25°C	26.0
Propylene Glycol, 10%, 25°C	0.06
Propylene Glycol, 50%, 25°C	0.9
Glycerin, 26°C	0.4
Peanut Oil, 25°C	1.4
Ether	33.3

C. Stability

Should be stored in tight containers.

VII. Analytical Methods

See Methylparaben

VIII. Occurrence

Propyl paraben is produced by esterifying p-hydroxybenzoic acid with n-propanol, using an acid catalyst such as sulfuric acid and an excess of propanol. The materials are heated in a glass lined reactor under reflux. The acid is then neutralized with caustic soda, and the product crystallized by cooling, centrifuged, washed, dried under vacuum, milled and blended, all in corrosion resistant equipment to avoid metallic contamination.

METHYL PARABEN

Biological Data

I. Acute Toxicity

Substance	Animal	Rt. of Admin.	Dosage (mg/kg)	Determination	Ref.
Methyl Paraben	Mouse	S.C.	333	LD_{50}	24
Methyl Paraben	Mouse	S.C.	125	LD_{50}	121
Methyl Paraben	Mouse	S.C.	1200	LD_{50}	2
Methyl Paraben	Rat	I.P.	960	LD_{50}	272
Methyl Paraben	Rat	P.O.	8000	LD_{50}	272
Methyl Paraben	Rabbit	P.O.	3000	LD_{100}	261
Methyl Paraben	Dog	P.O.	3000	LD_{100}	261

II. Short-Term Studies

Rats

Litters of young rats were divided into equal control and test groups. The test groups were fed 250 mg/kg or 500 mg/kg/day of methyl paraben for an unreported period of time. Growth of these rats was retarded, and the animals died during a cold spell when laboratory heating system failed. Despite repeated attempts, Bijlsma could not repeat these results (24).

Rabbits

Rabbits were fed methyl paraben at a dosage initially of 500 mg/kg/day for 6 days. On the 7th day through the 10th day, the level was raised 1000 mg/day. Rabbits were definitely affected by the 9th day and exhibited somnolence and ataxic motions within several hours of dosage with methyl paraben. Schuebel and Manger reported that the 3000 mg/kg level was toxic for these rabbits (262).

Cat

A white cat weighing 2.7 kg was fed methyl paraben at a dosage of 500 mg/kg/day for 6 days. The animal exhibited nausea, vomiting, and a general malaise within 15 minutes of dosage and remained in this condition throughout the duration of the 6 day dosage period (262).

Dogs

A dog fed 105 mg of methyl paraben/kg/day for 4 consecutive days showed no pathologic effects (24). Dogs fed methyl paraben at a dosage of 500 mg/kg/day for 7 days exhibited no toxic effects, but when this dosage was increased to 2000 or 3000 mg/kg/ they were affected (vomiting, tumors, etc.) (262).

Shuebel and Manger as well as most of the other workers reporting on the parabens tend to make statements that give the impression that conclusions on toxicity of these compounds are based on large numbers of observations. However, in the literature, data presented pertain to very few test subjects, frequently only a single animal. An additional problem in interpretation of some, if not most, of this literature is that results and procedures are not specified to the degree desirable to evaluate. This is particularly true of histological findings.

A group of 7 mongrel pups (5 test, 2 control) were housed individually and fed a dry diet ad lib supplemented with meat scraps. Six days weekly for 1 year, 3 of the dogs were given methyl paraben at a dosage of 1000 mg/kg/day and 2 were given 500 mg/kg/day in the form of a gelatin capsule. One month from the end of the experiment, blood samples were taken and determined for accumulation of methyl paraben (162).

No toxic symptoms were observed in any of the animals. All blood samples were normal. One of the females receiving 500 mg/kg/day was mated toward the end of the experiment and delivered a litter of healthy pups. All dogs were killed and autopsied. No abnormalities were found on macroscopic and microscopic examinations of the organs (162).

Man

Sabalitschka, et al., reported that a volunteer ingesting methyl paraben at a dosage of 2000 mg/day for 1 month was unaffected (249). Bracessi reported that he took 2000 mg of methyl paraben/day and concluded, after increasing this dosage over an indefinite period, that methyl paraben was essentially harmless to man (35).

III. Long-Term Studies

Rats

Two groups of 24 (12 male, 12 female) weanling Wistar rats were caged in groups of 4 and, upon maturity, separated so that there were only 2 animals/cage. Food and water were provided ad lib. One group was fed a stock diet, the other group a diet containing 2% methyl paraben. Food intake and weight were checked every 2 weeks (162).

After 40 weeks, no effects on growth were noted. This experiment was terminated after 96 weeks, and another experiment identical in set-up to the first (except for the inclusion of an 8% methyl paraben level) was initiated. This experiment also lasted 96 weeks (162).

Rats receiving 8% methyl paraben in the diet showed a slower rate of weight gain than did control animals. This difference was marked during the early part of the experiment and tended to dissappear later; the effect was more marked in males than in females. Animals receiving 2% methyl paraben were indistinguishable from controls. It would appear, according to Matthews, et al., that the toxic threshold for methyl paraben is at least 3000 mg/kg/day (162).

IV. Special Studies

Carcinogenic

A test group of 20 weanling female mice was administered 0.1 ml of a 1% methyl paraben solution in Carbowax 1000 (polyethylene glycol) twice weekly into the vagina by means of a syringe/blunt needle apparatus. A control group of 20 weanling mice were administered 0.1 ml of Carbowax 1000 similarly. The regimen was continued for 18 months, at which time all mice were sacrificed (34).

Eleven control animals survived 12 months, and 7 survived the full 18 months. Carcinomas were initiated in 5 mice. Of the methyl paraben-treated mice, 16 survived 12 months, and 8 survived the full 18 months. There were no carcinomas in any of these animals (34).

Other substances of known carcinogenic activity were also tested (DMBA). Although the test showed that carcinomas resulted from compounds not generally regarded as having carcinogenic activity, the significance is that methyl paraben did not initiate any carcinomas (34).

A carcinogenic study reported by Homburger which involved several techniques for ascertaining carcinogenicity, i.e., subcutaneous injection/secondary host transfer, intravenous injection/observation of lung adenomas, and co-carcinogenesis, did not show that methyl paraben had carcinogenic activity (121).

Sensitization

Ten male guinea pigs fed a stock diet supplemented with greens had hair removed (clipped) from back and flanks. A solution of 0.1% methyl paraben in physiological saline was prepared and injected 3 times weekly in the back and upper flanks. A total of 10 injections was given; the first injection was 0.5 ml; subsequent injections were 0.1 ml. Two weeks after the 10th injection a retest injection was made using 0.05 ml of a freshly prepared solution. Twenty-four hours later, the animals were observed for a reaction. There was no sensitization evident (162).

Methyl paraben was dissolved in propylene glycol in increasing concentrations and applied to the skin of 50 human subjects. Methyl paraben, when applied from 4-8 hours every other day for 10 applications, produced no irritation at the 5% level; however, higher concentrations than 5% did produce irritation (162).

Studies on the contact of aqueous solutions of methyl paraben with the conjunctiva showed that an aqueous solution less than 0.2% methyl paraben produced no effect in the rabbit. A 0.2% solution produced a slight, transitory conjunctival hyperemia (270).

In man, aqueous solutions of 0.1-0.3% produced a moderate hyperemia accompanied by slight lachrymation and a sensation of slight burning. These symptoms disappeared within approximately 1 minute. This regimen was repeated several times a day in more than 100 patients without resulting in any inconveniences to test subjects (270).

Schorr reports that, since 1966, there have been 8 cases of paraben allergy reported from the US in the scientific literature. The importance of parabens as a cause of long-standing, progressive, and severe iatrogenic contact dermatitis rests not in incidence of disease production but rather in the insidious pattern and ease with which paraben allergy can be misdiagnosed. Patients suffering from paraben allergy show cross-sensitivity to the 4 common paraben esters (methyl, ethyl, propyl, and butyl) when given a patch test (260).

Schamberg reports that an additional factor creating difficulty in recognizing paraben sensitivity is that the patch test may be negative to paraben-containing steroid creams as a result of the anti-inflammatory effect of corticosteroid. In Europe, the incidence of paraben sensitivity is much higher, possibly as a result of greater allowable concentration under their laws governing preservatives (255).

Biological Data

I. Acute Toxicity

Substance	Animal	Rt. of Admin.	Dosage (mg/kg)	Determination	Ref.
Propyl Paraben	Mouse	S.C.	1650	LD_{50}	2
Propyl Paraben	Rat	P.O.	8000	LD_{50}	272
Propyl Paraben	Rat	I.P.	640	LD_{50}	272
Propyl Paraben	Rabbit	P.O.	6000	LD_{100}	261
Propyl Paraben	Dog	P.O.	6000	LD_{100}	261

II. Short-Term Studies

Rabbits

Rabbits given propyl paraben per os at a dosage of 500 mg/kg/day for 6 days showed no ill effects. The first toxic effects appeared distinctly at a level of 3000 mg/kg/day (262).

Dogs

A dog administered propyl paraben daily in the diet over a 7 day period at increasing dosages from 1000-4000 mg/kg/day showed effects attributable to propyl paraben toxicity only at the 4000 mg/kg/ level (262).

A group of 6 mongrel pups (4 test, 2 control) were housed individually and fed a dry diet ad lib supplemented with meat scraps. Six days weekly for 1 year, 3 of the dogs were given 1000 mg of propyl paraben/ kg/day, and one was given 500 mg/kg/day in the form of a gelatin capsule. One month before the end of the experiment, blood samples were taken from the dogs at the 1000 mg/kg/day dosage and determined for accumulation of propyl paraben (162).

No toxic symptoms of any kind were observed. Blood samples revealed no trace of propyl paraben accumulation (162).

Man

Sabalitschka, et al., report that a volunteer ingesting propyl paraben at a dosage of 2000 mg of propyl paraben/man/day for 1 month resulted in no visible toxic effects (249).

III. Long-Term Studies

Rats

Two groups of 24 (12 male, 12 female) weanling Wistar rats were caged in groups of 4, and, upon maturity, separated so that there were only 2 animals to a cage. Food and water were provided ad lib. One group was

fed a stock diet, the other group a diet containing 2% propyl paraben. Food intake and weight was checked every 2 weeks (162).

After 40 weeks, no effects on growth were noted. The experiment was terminated after 96 weeks. Another experiment, identical to the first, was set up in which the dosage levels of paraben were 2 and 8%, and this experiment was also run for 96 weeks (162).

At the conclusion of each experiment, all animals were sacrificed, and kidney, liver, heart, lung, spleen, and pancreas tissues were removed for pathologic study. Rats dying during the course of the experiment were autopsied (162).

Animals receiving 8% propyl paraben in the diet showed a slower rate of weight gain than did controls. This difference was marked during the early phase of the experiment and tended to disappear later; it was also more noticeable in males than in females. Animals receiving the 2% dosage were indistinguishable from controls. Matthews opined that the first signs of toxicity would not be evident until an intake of at least 3000 mg/kg/day had been reached (162).

Propyl paraben fed to rats over an 18 month period at 150 mg/kg/day resulted in no ill effects and possibly some growth stimulation. At 1500 mg/kg/day growth depression resulted, but no irregular or pathologic changes could be found (272).

Weanling albino rats were divided into 4 test groups and were treated as follows: (8)

Group I — Forty rats were fed a mixture of 60 parts propyl paraben and 40 parts ethyl paraben at a level of 14/mg/kg/day for an 18 month study.

Group II — Twenty rats were fed this mixture at a level of 140 mg/kg/day for 18 months.

Group III — Twenty rats were fed this mixture at a level of 1400 mg/kg/day for 18 months.

Control — Twenty rats were fed a stock diet for 18 months.

At the end of 2 months, 10 group I animals were sacrificed for gross pathology, and 3 were selected for histologic study. At the end of 4 months, 10 additional rats were sacrificed, and 3 were examined histologically (lungs, liver, spleen, stomach, small intestine, kidney, and adrenals) (8).

Group 2, 3, and control animals were continued throughout the 18 month period. At the end of the experiment, all of the animals were sacrificed and 3 animals from each group were examined histologically (8).

All animals were weighed weekly, and gross pathologic and histologic examination results were recorded. The animals were fed the ethyl-propyl paraben mixture daily with the dosage level adjusted to their previous weekly weight (8).

The mixture resulted in an increase in growth rate at the 14 mg/kg/day and the 140 mg/kg/day level over the 18 month period. At the highest level, 1400 mg/kg/day, the mixture resulted in a growth depression by the 4th month. By the 8th month, growth rate of the rats at the highest dosage level was comparable with that of the control rats. This may have been indicative of a tolerance factor, or it may have been the result of age/metabolic change in the test animals (8).

Histological (pathological) findings revealed that, even at a level of about 1430 mg/kg/day for 18 months, there were no significant differences among test rats and control rats (8).

IV. Special Studies

Sensitization

Ten male guinea pigs fed a stock diet supplemented with greens had hair clipped from their backs and flanks. A solution of 0.1% propyl paraben (Na salt) in physiological saline was prepared and injected 3 times weekly into the back and/or upper flanks. A total of 10 random injections was given; the 1st injection was 0.05 ml, the remaining were 0.1 ml. Two weeks after the 10th injection, a retest injection was made using 0.05 ml of a freshly-prepared solution. Twenty-four hours later, the guinea pigs were observed for a reaction. No sensitization reaction to propyl paraben was observed (162).

Irritation Effect

Propyl paraben was dissolved in propylene glycol in increasing concentrations and applied to the skin of 50 human subjects. Propyl paraben, when applied from 4-8 hours every other day for 10 applications, produced no irritation at the 12% level; however, concentrations higher than 12% resulted in some irritation (162).

PARABENS

Biochemical Aspects

I. Breakdown

No information available from sources obtained.

II. Absorption-Distribution

Orally administered methyl and propyl paraben are readily and completely absorbed from the gastrointestinal tract. Matthews found that the use of the sodium salts appears to enhance absorption, since the fatal dose for the sodium salts is significantly lower than when the free ester is used (162).

III. Metabolism and Excretion

In vitro studies using esterases found in the liver and kidneys implicate those organs as the sites of paraben detoxication (136).

Oral administration of 100 mg of methyl or propyl paraben to rats (av. wt. 200 g) resulted in rapid detoxication, with metabolites appearing in the urine within one half hour after ingestion. Metabolites identified were p-hydroxybenzoic acid, p-hydroxyhippuric acid, an ester and ether glycuronides, and an ethereal sulphate. Methyl paraben was not detected in the urine. The concentration of phenolic acid in the blood remained extremely low throughout the experiment (71).

The metabolism of methyl and propyl paraben was investigated in rabbits by oral administration of a single dose of 0.4 or 0.8 g/kg. Thirty-nine percent of the administered methyl paraben was excreted as free p-hydroxybenzoic acid (PHBA), while the rest appeared as glycine (15%), glucuronic acid (ester- 7%, ether- 15%), and sulfuric acid (10%) conjugates of PHBA. Urinary excretion of the metabolites was rapid, 86% in 24 hours. With propyl paraben, 30% of the dose was excreted as free PHBA, while the glycine, glucuronic acid (ester and ether), and sulfuric acid conjugates of PHBA were found at levels of 24%, 7%, 13%, and 7% respectively. Seventy percent of the 0.4 g/kg dose of propyl paraben was excreted in 9 hours, 85% in 24 hours, and 88% in 48 hours. The rate of excretion was slightly lower with the larger dose (295, 296, 297).

In a study in which dogs were given, orally, 1.0 g methyl or propyl paraben/kg body wt/day for one year, between 96% and 100% of the daily administered dose was accounted for in the urine, chiefly in the form of conjugated end products (162).

Schuebel and Manger found that oral administration of methyl and propyl paraben to cats, rabbits, and dogs led to urinary excretion of 15-40% and 4-21%, respectively, of the dose in the form of free PHBA. No methyl or propyl paraben or free PHBA was excreted in the feces, indicating that the parabens were totally absorbed. It was proposed that the first step of paraben detoxication is saponification. This results in formation of PHBA, which is eliminated as such and in the form of salts through the kidneys. Part of this acid is combined with sulfuric acid or glycine. The authors suggest that most of this acid is decomposed in the body, the benzene ring being broken. The alcohol formed by the saponification may be of significance toxically if the rate of saponification is high enough; however, it is unlikely that enough paraben would be obtained from the diet to provide a significant quantity of alcohol. The authors found no evidence of accumulation of the parabens or their metabolites in the animal body (262).

Jones et al. found that dogs given a single oral dose of 1.0 g methyl paraben/kg/body weight excreted 66% of the administered dose in the urine within 24 hours (89% in 48 hrs), 21% as free PHBA and 33% as glucuronic acid conjugates. With a 1.0 g/kg dose of propyl paraben, 53% of the dose was excreted in the urine within 24 hours (58% in 48 hrs), 12% as free PHBA and 12% as glucuronic acid conjugates. The amount of free PHBA excreted never exceeded 0.5% of the dose. The free PHBA level of the plasma increased significantly after paraben administration, reaching a peak in approximately 6 hours and dropping back to zero within 48 hours. The high plasma and urine levels of free PHBA and conjugated products indicate hydrolysis of the ester linkage. No accumulation of methyl or propyl paraben or their metabolic products was noted in dogs which had been given 1.0 g/kg/day for one year. On the contrary, the rate of urinary excretion increased (to 96% of the dose after 24 hrs). Oral ingestion of 70 mg/kg/ by a fasted man revealed that methyl paraben is handled similarly in human metabolism. No detectable ester was found in the plasma or urine. After 12 hours, 50% of the administered dose was recovered from the urine of which 11% was accounted for as free PHBA (136).

Sabalitschka and Neufeld-Crzellitzer gave a human subject 2 g of propyl paraben daily for 5 days. They found that 17.4% of the administered dose was excreted in the form of PHBA, 13.7% free and 3.7% paired with glycine. They computed that about 55% of the propyl paraben was excreted paired with sulfuric acid. No propyl paraben could be found in the urine. The authors were unable to account for all of the administered paraben and, therefore, concluded that breaking of the benzene ring must occur to some extent (249).

IV. Effects on Enzymes and Other Biochemical Parameters

When 10-day old chick embryo femora were cultured for two days in medium containing methyl or propyl paraben, the result was increase in dry weight as compared to femora cultured in control medium. The methyl paraben concentrations found to stimulate growth were 10^{-5} and 10^{-6} M, propyl paraben concentrations were 10^{-5} to 10^{-7} M. It was suggested that these compounds may stabilize lysosomes (314).

In vitro studies have shown that methyl and propyl paraben are bound by protein and that this decreases their antimicrobial activity (1, 196). It is possible that protein-binding has similar effects on other aspects of paraben activity.

The action of methyl paraben on conduction in nerve fiber was studied in the cat. When applied directly to the spinal roots or to the vagus and sympathetic bundle in the neck as an 0.1 or 0.2% solution, conduction in nerve fibers of all sizes, myelinated and unmyelinated, was blocked, with smaller fibers being blocked first. Although the conduction block was reversible upon washing the nerve fiber, it is possible that some degradation occurred among the fibers. This could account for the prolonged or permanent relief, of pain, that is sometimes observed when a local anesthetic containing methyl paraben is injected in various painful states.

V. Drug Interaction

The antimicrobial effects of parabens and sodium benzoate are additive (50). It is possible that other aspects of the preservatives are similarly related.

Bubnoff et al. reported that i.v. and orally administered methyl paraben exhibits an anticonvulsive effect in rats with cocaine-induced cramps (39).

VI. Consumer Exposure Information

The major uses of the parabens in the food industry are cakes, pie crusts, pastries, icings, toppings and fillings (0.03-0.06% of a combination of 3:1 methyl and propyl parabens); soft drinks (0.03-0.05% of a 2:1 ratio of methyl and propyl parabens); creams and pastes (0.1% of a combination of parabens); jams, jellies, and preserves (0.07% of a 2:1 ratio of methyl and propyl parabens); olives and pickles (0.1% of a combination of parabens); and syrups (0.07%).

Under current legislation, the total addition of parabens as chemical preservatives is limited to 0.1% (50).

According to information compiled by the National Research Council the total amount of methyl paraben used in the United States in 1970 was 9,042 pounds and of propyl paraben, 4,031 pounds.

METHYL and PROPYL PARABEN

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M I C E

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FINAL REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date December 1, 1972

Laboratory No. 0901 r
Contract No. FDA 71-260

Sample: Fine white crystalline material

Marking: FDA 71-38 (Methyl paraben)

Examination Requested: Teratologic evaluation of FDA 71-38 in mice.

Procedure: See Appendix I

Results: See Tables 1 through 4 and Appendix II

Conclusion: Subject to reexamination in the light of additional data in progress, the following is concluded:

"The administration of up to 550 mg/kg (body weight) of the test material to pregnant mice for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

Comment: Attention is called to the fact that this is the sixteenth of a series of reports which will be issued in accordance with the terms of the contract cited above. Eventually, a total of at least 42 compounds will have been tested in 21 pairs; each pair being run concurrently against one sham-treated control and one positive control group. Because of the inherent variability of biological data of the type dealt with here, the accumulation and pooling of sequential sets of control values will greatly enhance the statistical value of the findings and the ultimate reliability of the test results.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgareidge

Kenneth Morgareidge, Ph.D.
Vice President

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups: 151 through 156

Material: FDA 71-38

Table 1

Fate Summary
(Mice)

Date: October 27, 1972

Laboratory No.: 0901 r

Group	Material	Dose mg/kg	Total		Surviving at Term	
			Mated	Pregnant	Total	Pregnant[1]
151	Sham	0.0	26	21	26	21
152	Aspirin*	150.0	30	22	29	21
153	FDA 71-38	5.5	26	21	25	20
154	FDA 71-38	25.5	27	25	23	21
155	FDA 71-38	118.0	24	21	23	20
156	FDA 71-38	550.0	31	23	29	21

* Positive Control: 150.0 mg/kg

** Administered as a water solution; 1.0 ml per kg of body weight; sham group dosed with corn oil

1) Includes all dams examined at term

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group: 151 through 156 | Table 2 | Date: October 27, 1972

Material: FDA 71-38 | Reproduction Data (Mice) | Laboratory No.: 0901 r

Group:	151	152	153	154	155	156
Dose (mg/kg):	Sham	Aspirin**	5.5	25.5	118.0	550.0
Pregnancies						
Total No.	21	22	21	25	21	23
Died or Aborted (before Day 17)	0	1	1	4	1	2
To term (on Day 17)	21	21	20	21	20	21
Corpora Lutea						
Total No.	365	400	370	379	322	397
Average/dam mated	14.0	13.3	14.2	14.0	13.4	12.8
Live Litters						
Total No.*	20	20	20	21	20	21
Implant Sites						
Total No.	259	246	266	255	235	248
Average/dam*	12.3	11.7	13.3	12.1	11.8	11.8
Resorptions						
Total No.*	25	39	12	11	13	14
Dams with 1 or more sites resorbed	10	14	9	8	9	10
Dams with all sites resorbed	1	1	--	0	--	--
Per cent partial resorptions	47.6	66.7	45.0	38.1	45.0	47.6
Per cent complete resorptions	4.76	4.76	--	--	--	--
Live Fetuses						
Total No.	230	197	248	240	219	229
Average/dam*	11.0	9.38	12.4	11.4	11.0	10.9
Sex ratio (M/F)	0.78	0.93	0.75	0.86	0.77	0.91
Dead Fetuses						
Total No.*	4	10	6	4	3	5
Dams with 1 or more dead	3	6	6	2	3	4
Dams with all dead	--	--	--	--	--	--
Per cent partial dead	14.3	28.6	30.0	9.52	15.0	19.1
Per cent all dead	--	--	--	--	--	--
Average Fetus Weight, g	0.89	0.82	0.91	0.84	0.86	0.88

* Includes only those dams examined at term.

** Positive Control: 150.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 151 through 156 — Laboratory No. 0901 r

Table 3

Material FDA 71-38 — Date October 27, 1972

Summary of Skeletal Findings* (Mice)

Findings / Group No. :	151	152	153	154	155	156
Dose (mg/kg):	Sham	Aspirin**	5.5	25.5	118.0	450.0
Live Fetuses Examined (at term)	159/20	136/20	171/20	170/21	155/20	161/21
Sternebrae						
Incomplete oss.	72/20	61/17	63/16	81/19	73/17	83/19
Scrambled						
Bipartite	3/3	6/5	2/2	5/5	9/6	1/1
Fused						
Extra						
Missing	19/7	39/11	23/7	30/12	32/9	5/5
Other						
Ribs						
Incomplete oss.						
Fused/split						
Wavy						
Less than 12						
More than 13	15/9	15/7	25/9	10/7	22/9	3/3
Other						
Vertebrae						
Incomplete oss.		2/1				
Scrambled						
Fused						
Extra ctrs. oss.						
Scoliosis						
Tail defects						
Other						
Skull						
Incomplete closure						
Missing						
Craniostosis						
Other						
Extremities						
Incomplete oss.		4/2	1/1	7/3	5/2	
Missing						
Extra						
Miscellaneous						
Hyoid; missing	45/13	54/17	31/10	43/17	52/16	27/11
Hyoid; reduced	23/15	17/12	14/9	20/12	17/9	17/10

* Numerator=Number of fetuses affected; Denominator=Number of litters affected.

** Positive control: 150.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 151 through 156 Date October 27, 1972

Material FDA 71-38 Laboratory No. 0901 r

Table 3-a
Summary of Soft Tissue Abnormalities
(Mice)

Group	Material	Dose Level mg/kg	Dam	Number of Pups	Description
151	Sham	0.0	S 1453	1	Meningoencephalocele
			S 1459	1	Meningoencephalocele
152	Aspirin*	150.0	A 1464	1	Meningoencephalocele
153	FDA 71-38	5.5	R 1011	1	Meningoencephalocele
154	FDA 71-38	25.5	R 1039	1	Meningoencephalocele
			R 1051	1	Meningoencephalocele
155	FDA 71-38	118.0	R 1064	1	Meningoencephalocele

* Positive Control: 150.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 151 through 156

Species Mice

Table 4

Average Body Weights*

Date October 27, 1972

Laboratory No. 0901 r

Group	Material	Dose Level	Day 0	6	11	15	17**
		mg/kg	g				
151	Sham	0.0	32.0	35.0	37.9	46.6	51.5 (21)
152	Aspirin***	150.0	30.7	33.7	35.4	41.8	46.9 (21)
153	FDA 71-38	5.5	32.5	34.3	38.1	47.9	54.6 (20)
154	FDA 71-38	25.5	28.6	30.4	35.2	43.1	50.6 (21)
155	FDA 71-38	118.0	29.7	32.8	35.7	45.0	51.1 (20)
156	FDA 71-38	550.0	30.5	33.1	36.8	44.2	48.9 (21)

* Of pregnant dams
** Number of surviving dams in parentheses (c.f. Table 1)
*** Positive control: 150.0 mg/kg

Appendix I

Teratology Study in Mice

Virgin adult female albino CD-1 outbred mice were individually housed in disposable plastic cages in temperature and humidity-controlled quarters with free access to food and fresh tap water. They were mated with young adult males, and observation of the vaginal sperm plug was considered Day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, the females were dosed with the indicated dosages by oral intubation; the controls were sham treated.

Body weights were recorded on Days 0, 6, 11, 15, and 17 of gestation. All animals were observed daily for appearance and behavior with particular attention to food consumption and weight, in order to rule out any abnormalities which may have occurred as a result of anorexic effects in the pregnant female animal.

On Day 17 all dams were subjected to Caesarean section under surgical anesthesia, and the numbers of implantation sites, resorption sites, and live and dead fetuses were recorded. The body weights of the live pups were also recorded. The urogenital tract of each dam was examined in detail for anatomical normality.

All fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses of each litter underwent detailed visceral examinations employing 10X magnification. The remaining two-thirds were cleared in potassium hydroxide (KOH), stained with alizarin red S dye and examined for skeletal defects.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 151

Material Sham

Dose 0.0 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date October 27, 1972

Laboratory No. 0901

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Sex		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead	M	F			
S 1451	P	15	14	13		5	8	1	0.86	
S 1452	NP	7	0						----	
S 1453	P	15	14	11		5	6	3	0.98	
S 1454	P	17	12	10		4	6	2	0.80	
S 1455	P	14	12	12		4	8		0.98	
S 1456	P	16	11	11		5	6		0.86	
S 1457	P	14	10	9		4	5	1	0.84	
S 1458	NP	11	0						----	
S 1459	P	16	13	13		5	8		0.93	
S 1460	NP	11	0						----	
S 1461	P	14	12	12		7	5		0.80	
S 1462	P	14	10	10		4	6		1.00	
S 1463	P	16	14	13		8	5	1	0.76	
S 1464	NP	10	0						----	
S 1465	P	18	15	12	2	5	7	1	0.85	
S 1466	P	18	15	14	1	6	8		0.74	
S 1467	P	13	7	3		2	1	4	0.97	
S 1468	P	14	13	11	1	5	6	1	0.99	
S 1469	NP	9	0						----	
S 1470	P	14	12	12		5	7		0.87	
S 1471	P	15	15	13		6	7	2	1.00	
S 1472	P	18	13	13		5	8		1.00	
S 1473	P	16	15	15		4	11		0.83	
S 1474	P	11	9					9	----	
S 1475	P	14	11	11		2	9		0.82	
S 1476	P	15	12	12		10	2		0.83	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 152

Material Aspirin

Dose 150.0 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date October 27, 1972

Laboratory No. 0901

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Sex		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead	M	F			
A 1451	P	16	11	8	1	3	5	2	0.95	
A 1452	P	14	7	5		1	4	2	0.93	
A 1453	P	16	15	13		6	7	2	0.80	
A 1454	NP	9	0						----	
A 1455	P	15	4	3		1	2	1	0.91	
A 1456	P	13	11	8	2	2	4	1	0.81	
A 1457	NP	11	0						----	
A 1458	P	16	13	11		3	8	2	0.82	
A 1459	P	15	13	10	2	4	6	1	0.79	
A 1460	P	16	12	12		6	6		0.88	
A 1461	P	12	10					10	----	Died Day 12
A 1462	P	15	10	1		0	1	9	0.64	
A 1463	P	14	7	6		5	1	1	0.98	
A 1464	P	14	12	9	3	4	5		0.79	
A 1465	P	17	15	14		9	5	1	0.87	
A 1466	NP	6	0						----	
A 1467	NP	7	0						----	
A 1468	NP	8	0						----	
A 1469	P	14	12	12		6	6		0.93	
A 1470	P	17	12	10		5	5	2	0.52	
A 1471	P	19	18	16	1	7	9	1	0.74	
A 1472	P	17	16	13		9	4	3	0.86	
A 1473	NP	10	0						----	
A 1474	NP	9	0						----	
A 1475	P	15	13	13		6	7		0.83	
A 1476	NP	12	0						----	
A 1477	P	12	11					11	----	

* P = Pregnant; NP = Not Pregnant

Continued on next page.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 152

Material Aspirin

Dose 150.0 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date October 27, 1972

Laboratory No. 0901

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Sex		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead	M	F			
Continued.										
A 1478	P	14	13	13		6	7		0.53	
A 1479	P	14	11	11		7	4		0.72	
A 1480	P	13	10	9	1	4	5		1.18	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 153

Material FDA 71-38

Dose 5.5 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date October 27, 1972

Laboratory No. 0901 r

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Sex		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead	M	F			
R 1001	P	11	12	11		4	7	1	0.98	
R 1002	NP	4	0						----	
R 1003	NP	13	0						----	
R 1004	NP	8	0						----	
R 1005	P	18	12	12		5	7		0.90	
R 1006	NP	7	0						----	
R 1007	P	16	12	12		6	6		0.94	
R 1008	P	20	3	3		2	1		1.29	
R 1009	P	15	13	12		5	7	1	0.87	
R 1010	P	15	11	10	1	2	8		0.87	
R 1011	P	15	12	10	1	7	3	1	0.91	
R 1012	NP	9	0						----	
R 1013	P	15	13	12		7	5	1	0.93	
R 1014	P	21	16	16		5	11		0.87	
R 1015	P	15	13	13		3	10		0.86	
R 1016	P	10	11				◦	11	----	Died Day 17
R 1017	P	16	15	15		7	8		0.95	
R 1018	P	16	15	13	1	5	8	1	0.95	
R 1019	P	15	16	13	1	4	9	2	0.88	
R 1020	P	17	15	12		3	9	3	0.89	
R 1021	P	17	14	14		7	6		0.98	
R 1022	P	17	16	16		8	8		0.96	
R 1023	P	13	14	12	1	6	6	1	0.71	
R 1024	P	17	16	15		6	9	1	0.67	
R 1025	P	15	15	14	1	5	9		0.81	
R 1026	P	15	13	13		9	4		0.94	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 154

Material FDA 71-38

Dose 25.5 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date October 27, 1972

Laboratory No. 0901 r

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Sex		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead	M	F			
R 1031	NP	3	0						----	
R 1032	P	27	11	11		4	7		0.79	
R 1033	P	16	15	13		7	6	2	0.93	
R 1034	P	19	16	16		7	9		0.82	
R 1035	P	15	10	9		4	5	1	0.77	
R 1036	P	14	11	10		3	7	1	0.65	
R 1037	P	15	10	9		5	4	1	0.81	
R 1038	P	15	14	13		7	6	1	0.88	
R 1039	P	14	11	11		6	5		0.90	
R 1040	P	16	12	12		5	7		0.89	
R 1041	NP	4	0						----	
R 1042	P	14	13	13		6	7		0.85	
R 1043	P	9	13		11			2	----	Died Day 15
R 1044	P	12	11					11	----	Died Day 16
R 1045	P	14	13	13		5	8		0.97	
R 1046	P	11	12					12	----	Died Day 14
R 1047	P	16	15	13		6	7	2	0.78	
R 1048	P	4	11					11	----	Died Day 17
R 1049	P	18	11	10	1	6	4		0.91	
R 1050	P	16	10	10		3	7		0.83	
R 1051	P	16	11	9		4	5	2	0.84	
R 1052	P	14	12	9	3	4	5		0.99	

Continued on next page.

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 154

Material FDA 71-38

Dose 25.5 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date October 27, 1972

Laboratory No. 0901 r

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Sex		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead	M	F			
Continued.										
R 1053	P	15	14	14		6	8		0.77	
R 1054	P	14	10	10		6	4		0.85	
R 1055	P	15	12	12		5	7		0.80	
R 1056	P	17	13	12		7	5	1	0.86	
R 1057	P	16	11	11		5	6		0.85	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 155

Material FDA 71-38

Dose 118.0 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date October 27, 1972

Laboratory No. 0901 r

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Sex		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead	M	F			
R 1061	P	14	10	10		3	7		0.93	
R 1062	NP	14	0						----	
R 1063	P	15	10	9		4	5	1	0.85	
R 1064	P	15	13	13		3	10		0.64	
R 1065	P	15	10	9	1	4	5		0.83	
R 1066	P	11	12					12	----	Died Day 15
R 1067	P	12	11	9		5	4	2	0.86	
R 1068	NP	4	0						----	
R 1069	P	15	11	11		5	6		0.82	
R 1070	P	15	13	13		5	8		0.91	
R 1071	P	13	12	10		4	6	2	0.83	
R 1072	P	14	14	12	1	3	9	1	0.99	
R 1073	P	19	14	14		7	7		0.85	
R 1074	P	16	13	13		7	6		0.87	
R 1075	P	13	10	9		3	6	1	0.89	
R 1076	P	13	11	11		6	5		0.88	
R 1077	P	12	11	8	1	4	4	2	0.73	
R 1078	P	15	12	12		5	7		0.97	
R 1079	P	14	10	10		3	7		0.88	
R 1080	P	13	11	9		6	3	2	0.78	
R 1081	NP	9	0						----	
R 1082	P	15	13	13		7	6		0.78	
R 1083	P	12	12	11		6	5	1	1.14	
R 1084	P	14	14	13		5	8	1	0.83	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 156

Material FDA 71-38

Dose 550.0 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date October 27, 1972

Laboratory No. 0901 r

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Sex		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead	M	F			
R 1091	P	15	13	12		5	7	1	0.84	
R 1092	NP	12	0						----	
R 1093	P	16	7					7	----	Died Day 15
R 1094										Not Assigned
R 1095	P	16	12	12		7	5		0.80	
R 1096	P	13	12	11		8	3	1	0.71	
R 1097	NP	4	0						----	
R 1098	P	15	13	10	2	5	5	1	0.78	
R 1099	P	13	12	11		6	5	1	0.89	
R 1100	NP	8	0						----	
R 1101	P	14	11	11		4	7		0.86	
R 1102	NP	8	0						----	
R 1103	P	17	14	12		4	8	2	0.80	
R 1104	P	15	14	12		5	7	2	0.97	
R 1105	P	13	14	12		6	6	2	0.90	
R 1106	P	16	15	14		7	7	1	0.75	
R 1107	P	13	13	12	1	4	8		0.82	
R 1108	NP	13	0						----	
R 1109	P	10	6	6		3	3		0.93	
R 1110	NP	11	0						----	
R 1111	NP	7	0						----	
R 1112	P	17	12	10		4	6	2	0.90	
R 1113	P	13	12	11		4	7	1	0.85	
R 1114	P	14	14	14		7	7		0.83	
R 1115	P	14	12	11	1	3	8		0.90	

Continued on next page.

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 156

Material FDA 71-38

Dose 550.0 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date October 27, 1972

Laboratory No. 0901 r

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Sex		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead	M	F			
Continued.										
R 1116	P	12	11	11		6	5		0.88	
R 1117										Not Assigned
R 1118	P	14	12	12		5	7		0.77	
R 1119	P	14	12	12		--	--		----	Died Day 10
R 1120	P	15	9	9		6	3		1.20	
R 1121	NP	9	0						----	
R 1122	P	13	11	11		5	6		1.09	
R 1123	P	13	6	5	1	5	0		0.92	

* P = Pregnant; NP = Not Pregnant

R A T S

Food and Drug Research Laboratories
INCORPORATED

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FINAL REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date December 1, 1972

Laboratory No. 0902 r
Contract No. FDA 71-260

Sample: Fine white crystalline material

Marking: FDA 71-38 (Methyl paraben)

Examination Requested: Teratologic evaluation of FDA 71-38 in rats

Procedure: See Appendix I

sults: See Tables 1 through 4 and Appendix II

Conclusion: Subject to reexamination in the light of later findings, the following is concluded:

"The administration of up to 550 mg/kg (body weight) of the test material to pregnant rats for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

Comment: Attention is called to the fact that this is the sixteenth of a series of reports which will be issued in accordance with the terms of the contract cited above. Eventually, a total of at least 42 compounds will have been tested in 21 pairs; each pair being run concurrently against one sham-treated control and one positive control group. Because of the inherent variability of biological data of the type dealt with here, the accumulation and pooling of sequential sets of control values will greatly enhance the statistical value of the findings and the ultimate reliability of the test results.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgareidge

Kenneth Morgareidge, Ph. D.
Vice President

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups: 151 through 156

Material: FDA 71-38

Table 1

Fate Summary

(Rats)

Date: October 27, 1972

Laboratory No.: 0902 r

Group	Material	Dose mg/kg	Total		Surviving at Term	
			Mated	Pregnant	Total	Pregnant[1]
151	Sham	0.0	24	23	24	23
152	Aspirin*	250.0	24	22	24	22
153	FDA-38	5.5	24	23	24	23
154	FDA-38	25.5	24	23	24	23
155	FDA-38	118.0	24	24	24	24
156	FDA-38	550.0	24	23	24	23

* Positive Control: 250.0 mg/kg

** Administered as a water solution; 1.0 ml per kg of body weight; sham group dosed with corn oil

1) Includes all dams examined at term

FOOD AND DRUG RESEAR LABORATORIES, INC.

Group: 151 through 156 | Table 2 | Date: October 27, 1972

Material: FDA 71-38 | Reproduction Data (Rats) | Laboratory No.: 0902 r

Group:	151	152	153	154	155	156
Dose (mg/kg):	Sham	Aspirin**	5.5	25.5	118.0	550.0
Pregnancies						
Total No.	23	22	23	23	24	23
Died or Aborted (before Day 20)	0	0	0	0	0	0
To term (on Day 20)	23	22	23	23	24	23
Corpora Lutea						
Total No.	277	278	292	281	295	287
Average/dam mated	11.5	11.6	12.2	11.7	12.3	12.0
Live Litters						
Total No.*	23	20	23	23	24	23
Implant Sites						
Total No.	249	258	280	272	296	275
Average/dam*	10.8	11.7	12.2	11.8	12.3	12.0
Resorptions						
Total No.*	7	39	8	11	11	8
Dams with 1 or more sites resorbed	4	9	6	8	5	5
Dams with all sites resorbed	--	2	--	--	--	--
Per cent partial resorptions	17.4	40.9	26.1	34.8	20.8	21.7
Per cent complete resorptions	--	9.09	--	--	--	--
Live Fetuses						
Total No.	241	218	272	261	285	267
Average/dam*	10.5	9.91	11.8	11.3	11.9	11.6
Sex ratio (M/F)	0.87	0.88	1.01	0.86	0.97	0.78
Dead Fetuses						
Total No.*	1	1	--	--	--	--
Dams with 1 or more dead	1	1	--	--	--	--
Dams with all dead	0	0	0	0	0	0
Per cent partial dead	4.35	4.55	--	--	--	--
Per cent all dead	--	--	--	--	--	--
Average Fetus Weight, g	3.67	2.21	3.64	3.66	3.82	3.82

* Includes only those dams examined at term.

** Positive Control: 250.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 151 through 156 — Laboratory No. 0902 r

Material FDA 71-38 — Date October 27, 1972

Table 3

Summary of Skeletal Findings*

(Rats)

Findings / Group No.	151	152	153	154	155	156
Dose (mg/kg):	Sham	Aspirin**	5.5	25.5	118.0	550.0
Live Fetuses Examined (at term)	163/23	145/20	179/23	173/23	192/24	176/23
Sternebrae						
Incomplete oss.	10/9	127/20	8/7	12/10	8/7	12/7
Scrambled						
Bipartite	1/1	4/3		1/1		1/1
Fused						
Extra						
Missing	1/1	89/18	1/1	2/2		3/1
Other						
Ribs						
Incomplete oss.	1/1	17/9				
Fused/split		5/4				
Wavy	8/4	52/16	7/6	3/2	9/4	17/8
Less than 12						
More than 13		78/17				
Other						
Vertebrae						
Incomplete oss.		122/20		1/1		2/2
Scrambled						
Fused		3/1				
Extra ctrs. oss.						
Scoliosis		9/2				
Tail defects						
Other						
Skull						
Incomplete closure	19/11	106/20	16/10	14/10	27/9	24/9
Missing		12/5				
Craniostosis						
Other						
Extremities						
Incomplete oss.		14/7				
Missing						
Extra						
Miscellaneous						
Hyoid; missing	11/7	91/20	9/5	6/5	18/10	5/4
Hyoid; reduced	3/3	4/4	3/2	1/1	3/2	8/4

* Numerator=Number of fetuses affected; Denominator=Number of litters affected.

** Positive control: 250.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 151 through 156 Date October 27, 1972

Material FDA 71-38 Laboratory No. 0902 r

Table 3-a
Summary of Soft Tissue Abnormalities
(Rats)

Group	Material	Dose Level mg/kg	Dam	Number of Pups	Description
152	Aspirin*	250.0	A 2453	1	Exencephaly; spina bifida
			A 2454	1	Exencephaly
			A 2456	2	Exencephaly; spina bifida
			A 2459	1	Exencephaly
			A 2468	6	Exencephaly; spina bifida
				1	Exencephaly

* Positive Control; 250.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 151 through 156 | Date October 27, 1972

Table 4

Species FDA 71-38 | Laboratory No. 0902 r

Average Body Weights*

Group	Material	Dose Level	Day 0	6	11	15	20**
		mg/kg	g				
151	Sham	0.0	232	251	269	285	355 (23)
152	Aspirin***	250.0	228	248	260	276	337 (22)
153	FDA 71-38	5.5	230	248	268	294	363 (23)
154	FDA 71-38	25.5	220	244	260	284	351 (23)
155	FDA 71-38	118.0	224	242	259	281	356 (24)
150	FDA 71-38	550.0	219	238	258	280	349 (23)

* Of pregnant dams
** Number of surviving dams in parentheses (c.f. Table 1)
*** Positive control: 250.0 mg/kg

Appendix I

Teratology Study in Rats

Virgin adult female albino rats (Wistar derived stock) were individually housed in mesh bottom cages in temperature and humidity-controlled quarters with free access to food and fresh tap water. They were mated with young adult males, and observation of the vaginal sperm plug was considered Day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, the females were dosed with the indicated dosages by oral intubation; the controls were sham treated.

Body weights were recorded on Days 0, 6, 11, 15, and 20 of gestation. All animals were observed daily for appearance and behavior with particular attention to food consumption and weight, in order to rule out any abnormalities which may have occurred as a result of anorexic effects in the pregnant female animal.

On Day 20 all dams were subjected to Caesarean section under surgical anesthesia, and the numbers of implantation sites, resorption sites, and live and dead fetuses were recorded. The body weights of the live pups were also recorded. The urogenital tract of each dam was examined in detail for anatomical normality.

All fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses of each litter underwent detailed visceral examinations employing 10X magnification. The remaining two-thirds were cleared in potassium hydroxide (KOH), stained with alizarin red S dye and examined for skeletal defects.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 151

Material Sham

Dose 0.0 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date October 27, 1972

Laboratory No. 0902

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Sex		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead	M	F			
S 2451	P	10	10	10		3	7		3.10	
S 2452	P	14	14	14		5	9		3.71	
S 2453	P	13	13	13		6	7		3.41	
S 2454	P	14	14	14		8	6		3.43	
S 2455	P	13	13	13		4	9		3.45	
S 2456	P	10	10	6		4	2	4	3.57	
S 2457	P	10	10	9		3	6	1	3.37	
S 2458	P	9	9	9		6	3		3.74	
S 2459	P	12	11	11		1	10		3.87	
S 2460	P	11	11	11		6	5		3.76	
S 2461	P	14	14	14		8	6		4.59	
S 2462	P	9	9	9		4	5		3.53	
S 2463	P	11	11	10		5	5	1	3.55	
S 2464	P	14	14	14		8	6		3.44	
S 2465	P	12	12	12		5	7		3.25	
S 2466	NP	11	0						----	
S 2467	P	10	10	9	1	5	4		3.39	
S 2468	P	9	1	1		1	0		4.30	
S 2469	P	13	13	13		9	4		3.82	
S 2470	P	11	3	3		2	1		4.97	
S 2471	P	9	9	9		3	6		3.78	
S 2472	P	13	13	13		6	7		3.29	
S 2473	P	10	10	9		4	5	1	3.53	
S 2474	P	15	15	15		6	9		3.53	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 152

Material Aspirin

Dose 250.0 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date October 27, 1972

Laboratory No. 0902

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Sex		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead	M	F			
A 2451	NP	11	0						----	
A 2452	P	12	12	12		5	7		2.18	
A 2453	P	12	12	12		7	5		1.75	
A 2454	P	12	12	12		2	10		2.45	
A 2455	P	11	11	10		6	4	1	2.79	
A 2456	P	13	13	13		4	9		2.51	
A 2457	P	13	13	10		4	6	3	2.44	
A 2458	P	13	13	13		7	6		2.45	
A 2459	P	11	11	10	1	3	7		2.02	
A 2460	P	12	12	10		5	5	2	2.55	
A 2461	P	13	13					13	----	
A 2462	P	6	6	6		3	3		2.80	
A 2463	P	15	15	15		8	7		2.09	
A 2464	P	11	11	10		6	4	1	1.98	
A 2465	P	12	13					13	----	
A 2466	P	10	10	10		6	4		2.27	
A 2467	P	15	15	13		5	8	2	2.10	
A 2468	P	14	14	11		6	5	3	1.73	
A 2469	P	13	13	12		7	5	1	2.65	
A 2470	P	11	11	11		6	5		2.99	
A 2471	NP	10	0						----	
A 2472	P	10	10	10		4	6		2.88	
A 2473	P	10	10	10		4	6		2.58	
A 2474	P	8	8	8		4	4		3.33	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 153

Material FDA 71-38

Dose 5.5 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date October 27, 1972

Laboratory No. 0902 r

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Sex		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead	M	F			
R 2001	P	12	13	12		3	9	1	3.13	
R 2002	P	13	8	8		4	4		3.59	
R 2003	P	7	7	6		3	3	1	4.33	
R 2004	P	12	12	11		2	9	1	3.29	
R 2005	P	17	17	17		10	7		3.32	
R 2006	P	12	12	12		4	8		3.41	
R 2007	P	13	13	13		5	8		3.37	
R 2008	P	12	12	12		6	6		3.61	
R 2009	P	9	9	8		4	4	1	3.75	
R 2010	P	13	13	13		9	4		3.58	
R 2011	P	11	11	11		7	4		3.06	
R 2012	P	13	13	13		7	6		3.38	
R 2013	P	12	12	12		7	5		3.58	
R 2014	P	15	15	15		11	4		3.61	
R 2015	P	14	14	14		7	7		3.78	
R 2016	P	10	10	9		7	2	1	3.72	
R 2017	P	14	14	14		9	5		3.53	
R 2018	NP	8	0						----	
R 2019	P	14	14	14		6	8		4.16	
R 2020	P	14	14	14		5	9		3.56	
R 2021	P	12	12	12		4	8		3.58	
R 2022	P	10	10	10		6	4		3.88	
R 2023	P	13	13	13		6	7		5.22	
R 2024	P	12	12	9		5	4	3	3.39	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 154

Material FDA 71-38

Dose 25.5 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date October 27, 1972

Laboratory No. 0902 r

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Sex		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead	M	F			
R 2031	P	6	6	2		2	0	4	2.40	
R 2032	P	15	15	14		8	6	1	3.64	
R 2033	P	17	17	17		10	7		4.24	
R 2034	P	12	12	12		5	7		4.05	
R 2035	P	11	11	11		5	6		3.87	
R 2036	P	11	11	10		6	4	1	3.52	
R 2037	P	8	8	8		4	4		3.95	
R 2038	P	12	12	12		8	4		3.42	
R 2039	P	10	10	9		4	5	1	3.54	
R 2040	P	11	11	11		4	7		3.58	
R 2041	P	12	12	12		5	7		3.62	
R 2042	P	14	14	14		5	9		3.89	
R 2043	P	11	11	10		7	3	1	3.62	
R 2044	P	14	14	13		6	7	1	3.71	
R 2045	NP	9	0						----	
R 2046	P	12	12	12		6	6		3.41	
R 2047	P	12	12	11		7	4	1	3.76	
R 2048	P	10	10	10		2	8		3.50	
R 2049	P	12	12	11		5	6	1	3.86	
R 2050	P	13	13	13		4	9		4.08	
R 2051	P	15	15	15		7	8		3.48	
R 2052	P	11	11	11		5	6		3.76	
R 2053	P	13	13	13		2	11		3.85	
R 2054	P	10	10	10		4	6		3.38	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 155

Material FDA 71-38

Dose 118.0 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date October 27, 1972

Laboratory No. 0902 r

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Sex		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead	M	F			
R 2061	P	13	13	13		5	8		3.66	
R 2062	P	13	13	13		10	3		3.93	
R 2063	P	13	13	13		10	3		3.80	
R 2064	P	11	11	11		6	5		3.88	
R 2065	P	15	15	14		9	5	1	3.79	
R 2066	P	13	13	13		2	11		3.96	
R 2067	P	12	12	7		3	4	5	3.76	
R 2068	P	15	15	14		7	7	1	3.94	
R 2069	P	11	11	11		6	5		3.75	
R 2070	P	13	13	13		6	7		3.57	
R 2071	P	11	11	11		5	6		4.25	
R 2072	P	14	14	14		4	10		3.69	
R 2073	P	10	10	9		5	4	1	3.86	
R 2074	P	11	12	9		6	3	3	3.64	
R 2075	P	15	15	15		10	5		3.59	
R 2076	P	15	15	15		6	9		3.21	
R 2077	P	13	13	13		6	7		3.38	
R 2078	P	12	12	12		3	9		3.77	
R 2079	P	8	8	8		4	4		3.79	
R 2080	P	10	10	10		5	5		3.63	
R 2081	P	14	14	14		6	8		5.58	
R 2082	P	11	11	11		2	9		3.58	
R 2083	P	10	10	10		7	3		4.35	
R 2084	P	12	12	12		7	5		3.39	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 156

Material FDA 71-38

Dose 550.0 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date October 27, 1972

Laboratory No. 0902 r

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Sex		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead	M	F			
R 2091	P	11	11	11		5	6		3.65	
R 2092	P	14	14	14		7	7		3.70	
R 2093	P	11	11	11		4	7		3.95	
R 2094	P	12	12	12		6	6		3.48	
R 2095	NP	11	0						---	
R 2096	P	12	12	10		5	5	2	4.06	
R 2097	P	11	11	11		5	6		3.55	
R 2098	P	13	13	13		9	4		4.02	
R 2099	P	11	11	9		3	6	2	3.83	
R 2100	P	15	15	15		8	7		3.57	
R 2101	P	12	14	14		5	9		4.67	
R 2102	P	8	8	7		3	4	1	4.47	
R 2103	P	14	14	14		9	5		3.36	
R 2104	P	14	14	14		8	6		3.83	
R 2105	P	9	9	7		4	3	2	3.64	
R 2106	P	13	13	13		6	7		3.91	
R 2107	P	14	14	14		6	8		3.52	
R 2108	P	8	8	8		4	4		4.71	
R 2109	P	12	12	12		3	9		3.78	
R 2110	P	17	14	14		4	10		3.53	
R 2111	P	12	12	11		3	8	1	3.73	
R 2112	P	10	10	10		2	8		3.67	
R 2113	P	12	12	12		4	8		3.78	
R 2114	P	11	11	11		4	7		3.48	

* P = Pregnant; NP = Not Pregnant

HAMSTERS

Food and Drug Research Laboratories
INCORPORATED

Maurice Avenue at 58th Street
Maspeth, New York 11378
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FINAL REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date December 1, 1972

Laboratory No. 0903 r
Contract No. FDA 71-260

Sample: Fine white crystalline material

Marking: FDA 71-38 (Methyl Paraben)

Examination Requested: Teratologic evaluation of FDA 71-38 in hamsters

Procedure: See Appendix I

sults: See Tables 1 through 4 and Appendix II

Conclusion: Subject to reexamination in the light of later findings, the following is concluded:

"The administration of up to 300 mg/kg (body weight) of the test material to pregnant hamsters for 5 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

Comment: Attention is called to the fact that this is the sixteenth of a series of reports which will be issued in accordance with the terms of the contract cited above. Eventually, a total of at least 42 compounds will have been tested in 21 pairs; each pair being run concurrently against one sham-treated control and one positive control group. Because of the inherent variability of biological data of the type dealt with here, the accumulation and pooling of sequential sets of control values will greatly enhance the statistical value of the findings and the ultimate reliability of the test results.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgareidge

Kenneth Morgareidge, Ph.D.
Vice President

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups: 151 through 156

Material: FDA 71-38

Date: October 27, 1972

Laboratory No.: 0903 r

Table 1

Fate Summary
(Hamsters)

Group	Material	Dose** mg/kg	Total		Surviving at Term	
			Mated	Pregnant	Total	Pregnant[1]
151	Sham	0.0	22	22	21	21
152	Aspirin*	250.0	22	22	21	21
153	FDA 71-38	3.0	22	22	22	22
154	FDA 71-38	14.0	22	21	22	21
155	FDA 71-38	65.0	22	22	21	21
156	FDA 71-38	300.0	25	21	25	21

* Positive Control: 250.0 mg/kg

** Administered as a water solution; 1.0 ml per kg of body weight; sham group dosed with corn oil

1) Includes all dams examined at term

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group: 151 through 156 | Table 2 | Date: October 27, 1972

Material: FDA 71-38 | Reproduction Data (Hamsters) | Laboratory No.: 0903 r

Group:	151	152	153	154	155	156
Dose (mg/kg):	Sham	Aspirin**	3.0	14.0	65.0	300.0
Pregnancies						
Total No.	22	22	22	21	22	21
Died or Aborted (before Day 14)	1	1	0	0	1	0
To term (on Day 14)	21	21	22	21	21	21
Corpora Lutea						
Total No.	320	326	339	324	343	343
Average/dam mated	14.6	14.8	15.4	14.7	15.6	13.7
Live Litters						
Total No.*	21	21	22	21	21	21
Implant Sites						
Total No.	245	233	250	237	246	242
Average/dam*	11.7	11.1	11.4	11.3	11.7	11.5
Resorptions						
Total No.*	10	6	15	2	4	11
Dams with 1 or more sites resorbed	6	6	7	2	3	7
Dams with all sites resorbed	0	0	0	0	0	0
Per cent partial resorptions	28.6	28.6	31.8	9.52	14.3	33.3
Per cent complete resorptions	--	--	--	--	--	--
Live Fetuses						
Total No.	234	227	235	235	241	231
Average/dam*	11.1	10.8	10.7	11.2	11.5	11.0
Sex ratio (M/F)	0.68	0.59	0.62	0.73	0.59	0.80
Dead Fetuses						
Total No.*	1	0	0	0	1	0
Dams with 1 or more dead	1	--	--	--	1	--
Dams with all dead	0	--	--	--	0	--
Per cent partial dead	4.76	--	--	--	4.76	--
Per cent all dead	--	--	--	--	--	--
Average Fetus Weight, g	1.81	1.82	1.89	1.84	1.85	1.93

* Includes only those dams examined at term.

** Positive Control: 250.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 151 through 156 — Laboratory No. 0903 r

Table 3

Material FDA 71-38 — Date October 27, 1972

Summary of Skeletal Findings*
(Hamsters)

Findings / Group No. :	151	152	153	154	155	156
Dose (mg/kg):	Sham	Aspirin**	3.0	14.0	65.0	300.0
Live Fetuses Examined (at term)	163/21	158/21	165/22	162/21	167/21	161/21
Sternebrae						
Incomplete oss.	100/21	96/21	85/22	114/21	90/21	85/21
Scrambled						
Bipartite	15/12	10/7	15/11	24/13	15/12	18/11
Fused	1/1					
Extra				2/2		1/1
Missing	22/11		8/6	20/10	8/5	15/4
Other						
Ribs						
Incomplete oss.						
Fused/split						
Wavy						
Less than 12						
More than 13	28/14	35/15	13/8	34/16	34/16	16/12
Other						
Vertebrae						
Incomplete oss.				3/2		
Scrambled						
Fused						
Extra ctrs. oss.						
Scoliosis						
Tail defects						
Other						
Skull						
Incomplete closure						
Missing						
Craniostosis						
Other						
Extremities						
Incomplete oss.	2/2			3/2	5/2	
Missing						
Extra						
Miscellaneous						
Hyoid; missing	2/2	1/1		2/2	1/1	
Hyoid; reduced		3/3	1/1	2/1	1/1	

* Numerator=Number of fetuses affected; Denominator=Number of litters affected.

** Positive control : 250.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 151 through 156 Date October 27, 1972

Material FDA 71-38 Laboratory No. 0903 r

Table 3-a
Summary of Soft Tissue Abnormalities
(Hamsters)

Group	Material	Dose Level mg/kg	Dam	Number of Pups	Description
151	Sham	0.0	S 3455	1	Medial rotation of hind limbs
152	Aspirin*	250.0	S 3467	1	Short hind limb

* Positive Control: 250.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 151 through 156 | Date October 27, 1972

Table 4

Species Hamsters | Laboratory No. 0903 r

Average Body Weights*

Group	Material	Dose Level	Day 0	6	8	10	14**
		mg/kg	g				
151	Sham	0.0	98.1	105.2	108.2	121.1	140.6 (21)
152	Aspirin***	250.0	98.9	106.9	110.3	121.6	141.7 (21)
153	FDA 71-38	3.0	98.8	105.1	109.1	121.1	141.5 (22)
154	FDA 71-38	14.0	97.1	103.0	105.6	116.7	136.0 (21)
155	FDA 71-38	65.0	99.2	106.0	109.3	121.0	140.5 (21)
156	FDA 71-38	300.0	97.8	104.0	108.0	119.5	140.6 (21)

* Of pregnant dams
** Number of surviving dams in parentheses (c.f. Table 1)
*** Positive control: 250.0 mg/kg

Appendix I

Teratology Study in Hamsters

Virgin adult female golden hamsters from an outbred strain were individually housed in mesh bottom cages in temperature and humidity controlled quarters with free access to food and fresh tap water at all times. They were mated (1 to 1) with mature males and the appearance of motile sperm in the vaginal smear was considered as Day 0 of gestation. Beginning on Day 6 and continuing daily through Day 10 of gestation, the indicated dose levels of the test material were administered by oral intubation; the controls were sham-treated.

Body weights were recorded on Days 0, 8, 10, and 14 of the gestation period. All animals were observed daily for appearance and behavior with particular attention to food consumption in order to better recognize any abnormalities resulting from anorexic effects in the pregnant animal.

On Day 14, all animals were subjected to Caesarian section under deep anesthesia and the numbers of implantation sites, resorption sites, live and dead fetuses were recorded. All live pups were weighed and the genital tract of each dam was examined for any anatomical abnormalities.

All fetuses were examined grossly for the presence of external congenital defects and one-third of each litter underwent detailed visceral examination under 10X magnification. The remaining two-thirds of the pups were cleared in potassium hydroxide, stained with alizarin red dye, and examined for the presence of sketal abnormalities.

FOOD AND DRUG RESEAI LABORATORIES, INC.

Group 151

Material Sham

Dose 0.0 mg/kg

Appendix II

Reproduction Data in Hamsters (Individual)

Date October 27, 1972

Laboratory No. 0903

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Sex		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead	M	F			
S 3451	P	11	11					11	----	Died Day 14
S 3452	P	15	10	8		3	5	2	1.92	
S 3453	P	15	13	13		4	9		1.92	
S 3454	P	14	10	10		4	6		1.90	
S 3455	P	17	15	15		3	12		1.79	
S 3456	P	16	14	14		6	8		2.07	
S 3457	P	16	13	13		4	9		1.64	
S 3458	P	14	11	10	1	4	6		1.92	
S 3459	P	16	11	11		5	6		1.50	
S 3460	P	13	10	10		3	7		1.73	
S 3461	P	15	12	12		5	7		1.45	
S 3462	P	13	10	8		4	4	2	1.73	
S 3463	P	16	12	12		5	7		1.62	
S 3464	P	12	10	9		3	6	1	1.60	
S 3465	P	10	9	8		3	5	1	1.92	
S 3466	P	15	12	12		6	6		1.97	
S 3467	P	11	10	7		4	3	3	2.04	
S 3468	P	16	14	14		7	7		1.93	
S 3469	P	16	12	11		5	6	1	1.71	
S 3470	P	16	12	12		5	7		2.00	
S 3471	P	17	13	13		6	7		1.84	
S 3472	P	16	12	12		6	6		1.92	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 152

Appendix II

Date October 27, 1972

Material Aspirin

Reproduction Data in Hamsters (Individual)

Laboratory No. 0903

Dose 250.0 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Sex		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead	M	F			
A 3451	P	14	8	8		3	5		1.86	
A 3452	P	12	9	9		2	7		2.02	
A 3453	P	15	9	9		2	7		1.91	
A 3454	P	16	11	11		3	8		1.62	
A 3455	P	15	13	13		4	9		1.83	
A 3456	P	19	12	11		4	7	1	1.88	
A 3457	P	14	12	12		4	8		----	Died Day 13
A 3458	P	15	13	13		4	9		1.85	
A 3459	P	14	12	11		3	8	1	1.61	
A 3460	P	16	14	14		5	8		1.82	
A 3461	P	17	12	12		4	8		1.51	
A 3462	P	16	10	10		5	5		1.79	
A 3463	P	16	14	14		4	10		1.83	
A 3464	P	15	10	9		2	7	1	1.83	
A 3465	P	16	11	11		4	7		1.79	
A 3466	P	15	12	12		9	3		1.95	
A 3467	P	11	8	7		3	4	1	1.71	
A 3468	P	16	13	13		5	8		1.85	
A 3469	P	15	13	12		5	7	1	2.00	
A 3470	P	13	10	9		5	4	1	1.83	
A 3471	P	11	7	7		3	4		1.92	
A 3472	P	15	12	12		5	7		1.82	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 153

Material FDA 71-38

Dose 3.0 mg/kg

Appendix II

Reproduction Data in Hamsters (Individual)

Date October 27, 1972

Laboratory No. 0903 r

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Sex		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead	M	F			
R 3001	P	15	11	11		3	8		1.89	
R 3002	P	17	10	10		2	8		1.87	
R 3003	P	15	8	8		3	5		1.82	
R 3004	P	14	12	12		4	8		1.84	
R 3005	P	15	11	5		1	4	6	2.41	
R 3006	P	17	9	9		3	6		1.79	
R 3007	P	16	12	12		4	8		1.75	
R 3008	P	14	11	10		2	8	1	1.82	
R 3009	P	18	14	14		8	6		1.74	
R 3010	P	17	12	12		5	7		1.83	
R 3011	P	14	10	10		1	9		1.59	
R 3012	P	13	11	9		2	7	2	1.92	
R 3013	P	15	10	10		3	7		1.84	
R 3014	P	16	11	11		4	7		1.94	
R 3015	P	14	11	11		8	3		1.90	
R 3016	P	12	11	11		4	7		1.85	
R 3017	P	15	12	11		5	6	1	1.84	
R 3018	P	16	13	13		6	7		2.01	
R 3019	P	16	11	10		5	5	1	2.09	
R 3020	P	19	15	15		7	8		2.09	
R 3021	P	14	12	10		4	6	2	1.89	
R 3022	P	17	13	11		6	5	2	2.00	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEAI LABORATORIES, INC.

Group 154 — Appendix II — Date October 27, 1972

Material FDA 71-38 — Reproduction Data in Hamsters (Individual) — Laboratory No. 0903 r

Dose 14.0 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Sex		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead	M	F			
R 3031	P	14	9	9		3	6		1.84	
R 3032	P	15	10	10		3	7		1.90	
R 3033	NP	12	0						----	
R 3034	P	17	13	13		5	8		1.71	
R 3035	P	18	11	11		5	6		1.84	
R 3036	P	17	12	12		4	8		1.77	
R 3037	P	13	10	10		5	5		1.69	
R 3038	P	19	17	17		4	13		1.67	
R 3039	P	15	13	13		4	9		1.60	
R 3040	P	15	12	11		4	7	1	1.66	
R 3041	P	15	10	9		4	5	1	1.76	
R 3042	P	14	9	9		3	6		1.75	
R 3043	P	12	10	10		3	7		1.74	
R 3044	P	11	9	9		5	4		2.04	
R 3045	P	15	13	13		7	6		1.89	
R 3046	P	14	11	11		6	5		1.94	
R 3047	P	15	10	10		6	4		1.80	
R 3048	P	11	9	9		4	5		1.83	
R 3049	P	15	12	12		6	6		2.11	
R 3050	P	17	12	12		5	7		2.11	
R 2051	P	15	12	12		6	6		1.91	
R 3052	P	15	13	13		7	6		2.05	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEAl) LABORATORIES, INC.

Group 156

Material FDA 71-38

Dose 130.0 mg/kg

Appendix II

Reproduction Data in Hamsters (Individual)

Date October 27, 1972

Laboratory No. 0903 r

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Sex		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead	M	F			
R 3091	P	13	10	10		3	7		2.09	
R 3092	P	15	12	12		4	8		1.65	
R 3093	P	16	11	10		2	8	1	1.93	
R 3094	P	19	13	13		5	8		1.91	
R 3095	P	18	15	14		6	8	1	2.05	
R 2096	P	19	13	13		4	9		1.85	
R 3097	P	16	11	11		6	5		1.82	
R 3098	P	17	15	15		5	10		1.84	
R 3099	P	15	12	12		7	5		2.12	
R 3100	P	17	16	14		10	4	2	1.81	
R 3101	P	17	11	11		6	5		1.80	
R 3102	NP	15	0						----	
R 3103	NP	7	0						----	
R 3104	P	15	12	11		6	5	1	2.01	
R 3105	P	11	10	10		4	6		1.79	
R 3106	NP	13	0						----	
R 3107	P	15	12	12		6	6		2.04	
R 3108	NP	8	0						----	
R 3109	P	14	11	11		6	5		2.05	
R 3110	P	11	8	8		3	5		2.10	
R 3111	P	12	10	9		4	5	1	1.90	
R 3112	P	13	9	9		6	3		2.16	
R 3113	P	8	8	8		4	4		1.92	
R 3114	P	8	12	8		2	6	4	2.17	
R 3115	P	11	11	10		4	6	1	1.81	

* P = Pregnant; NP = Not Pregnant

RABBITS

Food and Drug Research Laboratories
INCORPORATED

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FINAL REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date December 1, 1972

Laboratory No. 0904 r
Contract No. FDA 71-260

Sample: Fine white crystalline material

Marking: FDA 71-38 (Methyl Paraben)

Examination Requested: Teratologic evaluation of FDA 71-38 in rabbits

Procedure: (See Appendix I)

Results: (Data to Follow)

Conclusion:

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgareidge

Kenneth Morgareidge, Ph.D.
Vice President